PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

24 MAR 2005

Applicant's or agent's file reference N.89947 GCW	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04218	International filing date *(day/month/year)* 29.09.2003	Priority date *(day/month/year)* 27.09.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/86		
Applicant POWDERJECT RESEARCH LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.04.2004	15.02.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Schulz, R Telephone No. +31 70 340-4381

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04218

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-58 as originally filed

Claims, Numbers

1-44 received on 17.01.2005 with letter of 14.01.2004

Drawings, Sheets

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 31-36, 42, 43 with regard to industrial applicability

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 31-36, 42, 43 in part

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-44
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-44
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-30, 37-41,44
	No:	Claims	31-36, 42, 43

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1 Claims 32 - 36 and 43, as well as 31 and 42 in as far as they refer to *in vivo* expression, relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims according Art. 34(4)(a)(i) PCT.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2 **AMENDMENTS** (Art. 34(2) PCT)

2.1 The amendments filed with the letter dated 14.01.2004 do not introduce subject-matter which extends beyond the content of the application as filed and are thus allowable according to Art.34(2)(b) PCT.

3 **NOVELTY** (Art. 33 (1)(2) PCT)

3.1 The invention relates to a nucleic acid construct comprising at least two endogenous gene expression regulator units comprising each an endogenous promoter. These promoters are active at the same phase in viral life cycle and used for the co-expression of heterologous genes in mammalian cells. The construct may be further used as a multivalent vaccine and is assumed to display several advantages over the prior art (see p. 3, l. 15 - 25; p. 19, l. 7 - 18).

3.2 The following **documents** are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: FR 2715664 (Proteine performance)

D2: US 5162222 (Guarino, L.A. and Jarvis, D.L.)

D3: WO 98 30707 (Coffin, R.S.; Latchman, D.S.)

D4: van Drunen Littel-van den Hurk, S. et al. (2001) Immunization of livestock with DNA vaccines: current studies and future prospects. Vaccine 19, 2474 - 2479.

3.3 Claims 1 - 44 appear to be novel over the art in the sense of Art. 33 (1)(2) PCT because none of the documents cited discloses a nucleic acid construct as defined in claim 1, i.e. comprising ". . . an endogenous promoter capable of expression in a mammalian cell. . .":

3.4 D1 discloses a recombinant baculovirus comprising one or more expression cassette/s comprising a strong baculovirus promoter and a sequence coding for a heterologous gene. Preferred promoters are the p10 and the polyhedrine promoter (p.6, l. 7 - 10) which are active at the same phase of viral life cycle, i.e. very late in the infection cycle. The recombinant baculovirus constructs are designed for protein expression in insect cells.

3.5 D2 discloses recombinant baculoviruses for the expression of heterologous genes in insect cells during the immediate early phase of infection. The system comprises amongst others promoters from two different immediate early genes (column 3, l. 54 - column 4, l. 17; column 5, l. 19 - 35).

3.6 D3 discloses a nucleic acid construct comprising an expression cassette including the HSV LAT P2 region, a viral or a mammalian promoter and a heterologous gene, operably linked and used e.g. as a vaccine for the delivery of a mammalian cell (p. 3, l. 15 - 19). More than one of such an expression cassette of the invention could be introduced into a particular HSV strain, each comprising a heterologous gene (p. 4, l. 4 - 9).

3.7 D4 reviews strategies for the improvement of DNA vaccines in livestock animals referring several ways of delivery, such as e.g. to the gene gun approach (p. 2476, left column, 2nd para).

4 **INVENTIVE STEP** (Art. 33(1)(2) PCT)

4.1 The document D2 is regarded as being the closest prior art to the subject-matter of claim 1 and discloses recombinant baculoviruses for the expression of heterologous genes in **insect** cells during the immediate early phase of infection. The system comprises promoters from two different immediate early genes (column 3, l. 54 - column 4, l. 17; column 5, l. 19 - 35).

4.2 The subject-matter of claim 1 differs from this known plasmid vector in that the nucleic acid construct referred to comprises "at least two endogenous gene expression regulatory units which each comprise an endogenous promoter capable of expression in a **mammalian** cell. . .".

4.3 The problem to be solved by the present invention may therefore be regarded as the provision of an a recombinant viral vector construct for the co-expression of at least two heterologous genes in mammalian cells.

4.4 The proposed solution, is a recombinant construct for the expression of at least two heterologous genes under the control HSV endogenous gene expression regulatory units in mammalian cells.

4.5 In light of the closest prior art, the subject-matter of claims 1 - 44 is considered as comprising an inventive step and as to meet the requirements of Art. 33 (1)(3) PCT for the following reasons:

4.5.1 The invention disclosed in D2 is a vector for the improved expression of heterologous genes in insect cells, emphasising the importance of foreign gene expression during early stages of viral infection (column 2, l. 59 - column 3, l. 17).

4.5.2 Neither D2 nor any of the other documents cited suggested nor provided an incentive for the generation of a nucleic acid construct as defined in claim 1 for the expression of heterologous genes in mammalian cells.

5 COMMENT

5.1 For the assessment of the present claims 32 - 36 and 43, as well as 31 and 42 in as

far as they refer to *in vivo* expression, on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

6 CONCLUSION

6.1 Taken together, the present application is considered as to meet the requirements of Art. 33(1) PCT, because the subject-matter of claims 1 - 44 appears to be novel in the sense of Art.33 (2) PCT and involving an inventive step in the sense of Art.33 (3) PCT.

CLAIMS

1. A nucleic acid construct comprising viral genomic nucleic acid, said viral genomic nucleic acid comprising at least two endogenousgene expression regulatory units which each comprise an endogenous promoter capable of expression in a mammalian cell, where the endogenous promoters of the units are active at the same phase in the viral life cycle of the virus the viral genomic nucleic acid is derived from, where:

(a) at least two of the endogenous gene expression regulatory units comprising promoters active at the same phase are each operably linked to a separate heterologous coding sequence inserted into the viral genomic nucleic acid; and

(b) the viral genomic nucleic acid is from 1 to 50 kb in length excluding the heterologous sequences inserted into it.

2. A nucleic acid construct according to claim 1, wherein the at least two endogenous promoters are switched on at the same point in the viral life cycle of the virus the genomic nucleic acid is derived from.

3. A nucleic acid construct according to claim 1, wherein the at least two endogenous gene expression regulatory units are either both/all from immediate early or both/all from early viral genes.

4. A nucleic acid construct according to claim 1, wherein the at least two endogenous gene expression regulatory units are different.

5. A nucleic acid construct according to claim 1, wherein the virus the viral genomic nucleic acid is derived from is selected from the group consisting of a DNA virus and an RNA virus.

6. A nucleic acid construct according to claim 5, wherein the DNA virus is a double stranded DNA virus selected from a herpesvirus and an adeno associated virus (AAV).

7. A nucleic acid construct according to claim 6, wherein the herpesvirus is selected from the group consisting of a herpes simplex virus (HSV), a cytomegalovirus (CMV) and an Epstein Barr virus (EBV).

8. A nucleic acid construct according to claim 7, wherein the HSV is selected from the group consisting of HSV-1 and HSV-2.

9. A nucleic acid construct according to claim 7, wherein the viral genomic nucleic acid is derived from a herpes simplex virus and the at least two endogenous gene expression regulatory units each comprise an endogenous promoter selected from the group consisting of the ICP0, ICP4, ICP22 and ICP27 gene promoters.

10. A nucleic acid construct according to claim 7, wherein the viral genomic nucleic acid is derived from a herpes simplex virus and the two endogenous promoters of the at least two gene expression regulatory units are HSV tegument protein gene promoters.

11. A nucleic acid construct according to claim 7, wherein the viral genomic nucleic acid is from human cytomegalovirus and the endogenous promoters of the at least two gene expression regulatory units are:

- at least two selected from the group consisting of the UL36, UL37 and UL38 gene promoters;
- the UL82 and UL83 gene promoters; or
- the UL122 and U123 gene promoters.

12. A nucleic acid construct according to claim 1, wherein all of the heterologous coding sequences expressed by the endogenous gene expression regulatory units are derived from the same organism.

13. A nucleic acid construct according to claim 1, wherein two or more of the heterologous coding sequences encode antigens.

14. A nucleic acid construct according to claim 1, wherein the antigens are antigens from a pathogen.

15. A nucleic acid construct according to claim 1, wherein some or all of the viral sequences, apart from the at least two endogenous gene expression regulatory units, which are present in the region of the viral genome corresponding to that between the 5' and 3' ends of the viral genomic nucleic acid in the construct are absent from the construct.

16. A nucleic acid construct according to claim 15, wherein the absent region comprises part or all of the intervening sequences between two of the adjacent endogenous gene expression regulatory units linked to heterologous coding sequences.

17. A nucleic acid construct according to claim 15, wherein the absent region corresponds to one or more of the genes present in the region of the viral genome other than those of the at least two endogenous gene expression regulatory units used to express the heterologous coding sequences.

18. A nucleic acid construct according to claim 15, wherein the viral genomic nucleic acid is from HSV-2 and the viral sequences have been removed from the construct by one or more of the following techniques:

(a) a partial digestion with a BstXI enzyme and then religation to remove sequences between ICP27 and ICP0;

(b) a complete digestion with a BspHI enzyme, followed by a partial digestion with a BsiWI enzyme and then religation to remove sequences adjacent to ICP22;

(c) a digestion with a SrfI enzyme and then religation to remove sequences between ICP4 and ICP0; and

(d) total digestion with a BstXI enzyme and then religation to remove sequences beween ICP27 and ICP0.

19. A nucleic acid construct according to claim 15, wherein the viral genomic nucleic acid is from HSV-1 and the viral sequences have been removed from the construct to remove substantially all of the HSV-1 sequences extraneous to ICP0, ICP4, ICP22 and ICP27 coding sequences.

20. A nucleic acid construct according to claim 1, wherein the viral genomic nucleic acid corresponds to a contiguous region of the viral genome it is derived from apart from the replacement of the coding sequences the endogenous gene expression regulatory units are naturally operably linked to with the heterologous coding sequences.

21. A nucleic acid construct according to claim 1, wherein the

endogenous gene expression regulatory units operably linked to the heterologous coding sequences are endogenous promoters.

22. A method of generating a nucleic acid construct for direct administration to a subject to elicit an immune response in the subject, the method comprising:

(a) inserting viral genomic nucleic acid into a vector backbone, said viral genomic nucleic acid comprising at least two endogenous gene expression regulatory units which each comprise an endogenous promoter capable of expression in a mammalian cell, where the endogenous promoters of the units are active at the same phase in the viral cycle of the virus the viral genomic nucleic acid is derived from; and

(b) either prior to, at the same time, or subsequent to inserting the viral genomic nucleic acid into the vector backbone, operably linking each of the endogenous promoters of at least two of the gene expression regulatory units in the viral genomic nucleic acid to heterologous coding sequences

wherein the viral genomic nucleic acid is from 1 to 50 kb in length excluding the heterologous sequences inserted into it.

23. A method according to claim 22, wherein the method further comprises deleting from the viral genomic nucleic acid some or all of the viral sequences, apart from the at least two endogenous gene expression regulatory units, which are present in the region of the viral genome corresponding to that between the 5' and 3' ends of the viral genomic nucleic acid of the construct.

24. A method according to claim 23, wherein the deleted sequences are some or all of the non-coding intervening sequences between adjacent endogenous gene expression regulatory units to which the heterologous coding sequences are to be operably linked.

25. A method according to claim 22, wherein the genomic nucleic acid is inserted into the vector backbone as a single fragment.

26. Coated particles, suitable for delivery from a particle-mediated delivery device, which particles comprise carrier particles coated with a nucleic acid

construct wherein the construct comprises viral genomic nucleic acid, said viral genomic nucleic acid comprising at least two endogenous gene expression regulatory units which each comprise an endogenous promoter capable of expression in a mammalian cell, where the endogenous promoters of the units are active at the same point in the viral cycle of the virus the viral genomic nucleic acid is derived from, where:

- at least two of the endogenous gene expression regulatory units comprising gene expression regulatory units comprising promoters are each operably linked to a heterologous coding sequence inserted into the viral genomic nucleic acid; and
- the viral genomic nucleic acid is from 1 to 50 kb in length excluding the heterologous sequences inserted into it.

27. Coated particles according to claim 26, wherein the carrier particles are gold or tungsten.

28. A dosage receptacle for a particle mediated delivery device comprising coated particles according to claim 26.

29. A particle mediated delivery device loaded with coated particles according to claim 26.

30. A particle mediated delivery device according to claim 29 which is a needleless syringe.

31. A method of obtaining expression in a mammalian cell of a polypeptide of interest, which method comprises transferring into said cells a nucleic acid construct comprising viral genomic nucleic acid, said viral genomic nucleic acid comprising at least two endogenous gene expression regulatory units which each comprise an endogenous promoter capable of expression in a mammalian cell, where the endogenous promoters of the units are active at the same phase in the viral cycle of the virus the viral genomic nucleic acid is derived from, where:

- at least two of the endogenous gene expression regulatory units comprising promoters are each operably linked to a heterologous coding sequence inserted into the viral genomic nucleic acid; and

- the viral genomic nucleic acid is from 1 to 50 kb in length excluding the heterologous sequences inserted into it.

32. A method according to claim 31, wherein the construct is delivered directly into a subject.

33. A method according to claim 32, wherein the construct is delivered by injection, transdermal particle delivery, inhalation, topically, orally, intranasally or transmucosally.

34. A method according to claim 32, wherein the construct is delivered by needleless injection.

35. A method according to claim 34, wherein the nucleic acid construct is coated onto carrier particles.

36. A method of nucleic acid immunisation comprising administering to a subject an effective amount of coated particles, which particles are suitable for delivery from a particle-mediated delivery device, the particles comprising carrier particles coated with a nucleic acid construct, wherein the construct comprises viral genomic nucleic acid, said viral genomic nucleic acid comprising at least two endogenous gene expression regulatory units which each comprise an endogenous promoter capable of expression in a mammalian cell, where the endogenous promoters of the units are active at the same phase in the viral cycle of the virus the viral genomic nucleic acid is derived from, where:

- at least two of the endogenous gene expression regulatory units comprising promoters are each operably linked to a heterologous coding sequence inserted into the viral genomic nucleic acid; and
- the viral genomic nucleic acid is from 1 to 50 kb in length excluding the heterologous sequences inserted into it.

37. A method of generating a nucleic acid construct for direct administration to a subject to elicit an immune response in the subject, the method comprising:

(a) inserting viral genomic nucleic acid into a vector backbone, said viral genomic nucleic acid comprising at least two endogenousgene expression

regulatory units which each comprise an endogenous promoter capable of expression in a mammalian cell, where the endogenous promoters of the units are active at the same phase in the viral cycle of the virus the viral genomic nucleic acid is derived from; and

(b) either prior to, at the same time, or after inserting the viral genomic nucleic acid into the vector backbone, deleting from the viral genomic nucleic acid some or all of the viral sequences, apart from the at least two endogenous gene expression regulatory units, which are present in the region of the viral genome corresponding to that between the 5' and 3' ends of the viral genomic nucleic acid of the construct

where the length of the viral genomic nucleic acid inserted into the vector backbone being from 1 to 50 kb.

38. A method according to claim 37, wherein the nucleic acid sequences deleted are part or all of the non-coding intervening sequences between two of the endogenous promoters.

39. Coated particles, suitable for delivery from a particle-mediated delivery device, which particles comprise carrier particles coated with a nucleic acid construct generated by a method as defined in claim 36.

40. A dosage receptacle for a particle mediated delivery device comprising coated particles according to claim 39.

41. A particle mediated delivery device loaded with coated particles according to claim 40.

42. A method of obtaining expression in a mammalian cell of a polypeptide of interest , which method comprises transferring into said cells a nucleic acid construct generated by a method according to claim 37.

43. A method of nucleic acid immunisation comprising administering to a subject an effective amount of coated particles, which particles are suitable for delivery from a particle-mediated delivery device, the particles comprising carrier particles coated with a nucleic acid construct generated by a method according to claim 37.

44. Use of a nucleic acid construct according to any one of claims 1 to 21, a nucleic acid construct generated by a method according to any one of claims 22 to 25, 37 and 38 or coated particles according to any one of claims 26, 27, and 39 in the manufacture of a medicament for use in nucleic acid immunisation.

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